

# Human recombinant interleukin-1 receptor antagonist inhibits lymphocyte blastogenesis induced by concanavalin A

## Restorative effect of hrIL-1

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Interleukin-1 (IL-1), mainly produced by monocyte-macrophages, is a polypeptide cytokine with pleiotropic biological effects. IL-1 plays an important role in mediating immune response and inflammation. Recently a natural inhibitor to IL-1 has been discovered, interleukin-1 receptor antagonist (IL-1ra), produced by human monocytes cultured on adherent IgG which binds to the IL-1 receptors. In our study we found that the pretreatment of cells with serial dilutions of IL-1ra (250 ng/ml–2.5 pg/ml) inhibits, in a dose-dependent manner, lymphocyte DNA synthesis stimulated with Con A (10 µg/ml). IL-1ra did not have any effect on resting peripheral blood mononuclear cells (PBMC). Time course experiments show that IL-1ra at 250 ng/ml has its maximum inhibitory effect on lymphocyte blastogenesis when cells are pretreated 2 h before Con A. No effect was found when hrIL-1ra was added after Con A. Moreover, hrIL-1ra also inhibits the enhancing effects of exogenous hrIL-1 (400, 200, 100 and 50 ng/ml) on lymphocytes stimulated with Con A; while when hrIL-1ra was used on cells treated with only Con A, the inhibition was more pronounced. When PBMC were removed from monocytes, by adherence, the Con A-treated lymphocytes were not influenced by 2 h pretreatment of hrIL-1ra; while a strong inhibition was found when exogenous hrIL-1 was added at different concentrations. In addition, hrIL-1ra also inhibits the enhancing effect of hrIL-2 on lymphocyte DNA synthesis. In another set of experiments PBMC were pretreated with hrIL-1ra (250 ng/ml) for 2 h and then added LPs (10 ng/ml) and IL-1α generation was determined using ELISA. In these experiments IL-1ra completely abolished the generation of IL-1α. These data suggest that hrIL-1ra exhibits a dose-response inhibition of lymphocyte blastogenesis induced by Con A, probably through the down-regulation of IL-1 synthesis necessary as an early signal for T-cell activation and IL-2 production.

Interleukin-1 receptor antagonist; Concanavalin A; Lymphocyte; Blastogenesis; Interleukin; T-cell

## 1. INTRODUCTION

Interleukin-1 (IL-1) is a cytokine polypeptide product called monokine with a broad range of biological actions, including the regulation of the immune system [1–5]. This monokine is generated by a variety of cell types including monocyte-macrophage cells, which play important roles in several phases of the immune response to both foreign (including mitogens) and some self antigens [6,7]. It has been reported by many authors that the role of the antigen in activating macrophages for the production of monokines and T-cell activation is very critical to the host's immune response [8].

**Abbreviations:** hrIL-1ra, human recombinant interleukin-1 receptor antagonist; hrIL-1, human recombinant interleukin-1; IL-2, interleukin-2; Con A, concanavalin A; PBMC, peripheral blood mononuclear cells; <sup>3</sup>H-TdR, [*methyl*-<sup>3</sup>H]thymidine

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Recently, two independent groups have described a natural inhibitor to IL-1 [9–11]. This protein which binds to the IL-1 receptor, is called IL-1 receptor antagonist and has been proved to have interesting biological properties [12,13]. Human recombinant interleukin-1 receptor antagonist (hrIL-1ra) is a monokine secreted by macrophages with a chemical structure similar to hrIL-1β but with different biological activity. We have recently found that hrIL-1β and IL-1α have similar biological effects on natural killer cell activity in potentiating the stimulatory effects of IL-2 [14]. Moreover, hrIL-1ra inhibits IL-1α and IL-1β stimulation of PGE2 production in human synovial cells [12]. Various *in vitro* T lymphocyte functions are initiated or potentiated by contact with specific lymphokines. Resting peripheral blood T lymphocytes can be activated to proliferate after interacting with polyclonal mitogens or antigens. IL-1 is the first signal inducing the expression of IL-2 receptors on the surface of a subpopulation of T cells and the binding of IL-1 to its receptor leads the cells to initiate DNA synthesis [15], a process mediated by the binding of IL-2 to

its receptor. Since there are many immunological changes attributed to hrIL-1, including its effects on the augmentation of T cell proliferation to mitogen and antigens, a property known as lymphocyte activating factor (LAF) [16], it is pertinent to study the effects of hrIL-1ra on lymphocyte blastogenesis induced by a mitogen, concanavalin A (Con A) and its influence on LPS stimulated peripheral blood mononuclear cell production of IL-1 $\alpha$ .

## 2. MATERIALS AND METHODS

### 2.1. Peripheral blood mononuclear cells (PBMC) preparation

Cell suspensions were prepared from heparinized peripheral blood of healthy volunteers by using a gradient centrifugation of Fycoll-Hypaque [17] (Pharmacia Fine Chemicals, Piscataway, NJ, USA). The mononuclear cells that accumulated at the interface between the separating medium and the plasma were removed, washed three times and resuspended in RPMI 1640 (Gibco) medium. The mononuclear cell suspension contained 80–85% lymphocytes and 15–20% monocytes. These cells were diluted to a concentration of  $2.5 \times 10^5$  cells/ml in RPMI 1640 medium containing heat-inactivated (30 min at 56°C), 10% FCS, as well as HEPES (25 mM), penicillin G (200 U/ml), gentamicin (10  $\mu$ g/ml), L-glutamine (0.3 mg/ml), this will be referred to as complete medium (CRPMI). The cells were incubated for 72 h at 37°C in a 5% CO<sub>2</sub>-humidified atmosphere in multiwell plates (Falcon, Italy), with or without Con A 10  $\mu$ g/ml (Sigma, St. Louis, MO, USA). After 66 h, 2  $\mu$ Ci of [<sup>3</sup>H]thymidine was added to each well and the incubation was continued for 6 h at 37°C.

The uptake of [<sup>3</sup>H]thymidine was quantified by trapping and washing the lymphocytes on glass filter in a Skatron cell harvester (Flow Laboratories, Milano, Italy) and counting the radioactivity on glass fiber filters (Flow Lab.). The results were expressed as the mean counts per min (cpm)  $\pm$  SD, and the percentage of inhibition of T lymphocyte uptake of [<sup>3</sup>H]thymidine by hrIL-1ra was calculated from the net uptake of radioactivity of stimulated T lymphocytes in the presence of hrIL-1ra, at different concentrations, to that of the absence of hrIL-1ra. Antiserum anti-IL-1 $\beta$  was added at different concentrations 30 min before adding the mitogen Con A (Sigma) or 200 ng/ml of normal rabbit serum (NRS) used as a control.

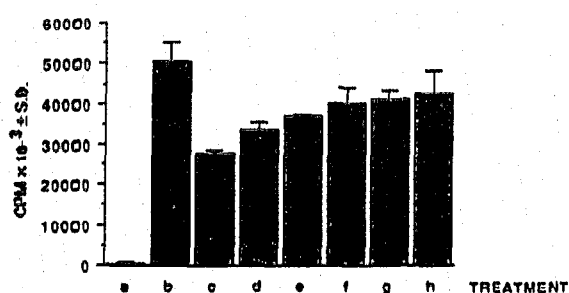


Fig. 1

a=Nil  
b=Con A 10  $\mu$ g/ml  
c=hrIL-1ra 250 ng/ml + Con A 10  $\mu$ g/ml  
d=hrIL-1ra 25 ng/ml + Con A 10  $\mu$ g/ml  
e=hrIL-1ra 2.5 ng/ml + Con A 10  $\mu$ g/ml  
f=hrIL-1ra 0.25 ng/ml + Con A 10  $\mu$ g/ml  
g=hrIL-1ra 0.025 ng/ml + Con A 10  $\mu$ g/ml  
h=hrIL-1ra 0.0025 ng/ml + Con A 10  $\mu$ g/ml

Fig. 1. The histogram represents the c.p.m. of <sup>3</sup>H-TdR incorporation by lymphocytes stimulated with Con A (10  $\mu$ g/ml) for 72 h and pretreated with serial concentrations of IL-1ra for 2 h. The data are representative of 4 experiments and the values represent the mean  $\pm$  SD of triplicate determinations.

[methyl-<sup>3</sup>H]Thymidine (<sup>3</sup>H-TdR) (specific activity 25 Ci/mmol), was purchased from New England Nuclear, Boston, MA, USA. hrIL-1ra (Upjohn Co., Kalamazoo, MI) was a kind gift from Dr Daniel Tracey. hrIL-2 was purchased from Cetus Co. (Emeryville, CA, USA).

### 2.2. ELISA for IL-1 $\alpha$

PBMC ( $2.5 \times 10^6$ /ml) were cultured for 24 h with and without Con A. The levels of IL-1 $\alpha$  in PBMC culture supernatants were determined by a cytokine-specific ELISA obtained from ENDOGEN Inc. (Boston, MA). The kit is composed of 96 determinations and the assays were performed exactly as recommended by the manufacturer.

The ELISA reader was set at 405 nm absorbance and all the samples were read after 1 h incubation at room temperature. The standard curve was constructed by plotting the mean absorbance of the interleukin standard dilutions against the concentration of the interleukin standards. By interpolation from the absorbance values (Y axis), the interleukin values (X axis) were determined by comparison to the standard curves. All samples were assayed in triplicate. Results are expressed as pg/ml ( $\pm$  SD).

LPS was purchased from Sigma and was used at a concentration of 10 ng/ml.

## 3. RESULTS

Peripheral blood mononuclear cell mitogen stimulation is a good model to study the induction of DNA synthesis and its inhibition by many antagonists. In addition, it has been suggested that IL-1 influences and potentiates the function of certain T lymphocyte proliferation [18,19,25]. In Fig. 1 we show the inhibition of DNA synthesis which occurs in PBMC when pretreated

Table I

| Treatment  | <sup>3</sup> H-TdR c.p.m. $\pm$ SD | P <   |
|--|------------------------------------|-------|
| Control  | 1099 $\pm$ 96                      | —     |
| Con A 2 $\mu$ g  | 21834 $\pm$ 2006                   | —     |
| Con A 10 $\mu$ g   | 50226 $\pm$ 2561                   | (*)   |
| Con A 50 $\mu$ g   | 32790 $\pm$ 1099                   | —     |
| (1) hrIL-1ra 250 ng/ml + Con A 10 $\mu$ g/ml (hrIL-1ra and Con A are incubated at the same time) | 44177 $\pm$ 2038                   | 0.05  |
| (2) hrIL-1ra 250 ng/ml + Con A 10 $\mu$ g/ml (hrIL-1ra is incubated 30 min before Con A)         | 35452 $\pm$ 1601                   | 0.005 |
| (3) hrIL-1ra 250 ng/ml + Con A 10 $\mu$ g/ml (hrIL-1ra is incubated 2 h before Con A)            | 25979 $\pm$ 2031                   | 0.001 |
| (4) hrIL-1ra 250 ng/ml + Con A 10 $\mu$ g/ml (hrIL-1ra is incubated overnight before Con A)      | 26637 $\pm$ 1346                   | 0.001 |
| (5) Con A 10 $\mu$ g/ml + hrIL-1ra 250 ng/ml (hrIL-1ra is incubated 2 h after Con A)             | 47459 $\pm$ 2189                   | N.S.  |
| (6) Con A 10 $\mu$ g/ml + hrIL-1ra 250 ng/ml (hrIL-1ra is incubated 66 h after Con A)            | 51805 $\pm$ 4901                   | N.S.  |

This time-course table represents the c.p.m. of <sup>3</sup>H-TdR incorporation by lymphocytes stimulated with Con A for 72 h and treated with IL-1ra (250 ng/ml) at different times before and after Con A. The PBMC were treated with different concentrations of Con A. The data are representative of 4 experiments and the values represent the mean  $\pm$  SD of triplicate determinations. P values (Student's t-test) are calculated by comparing Con A 10  $\mu$ g/ml (\*) with hrIL-1ra + Con A.

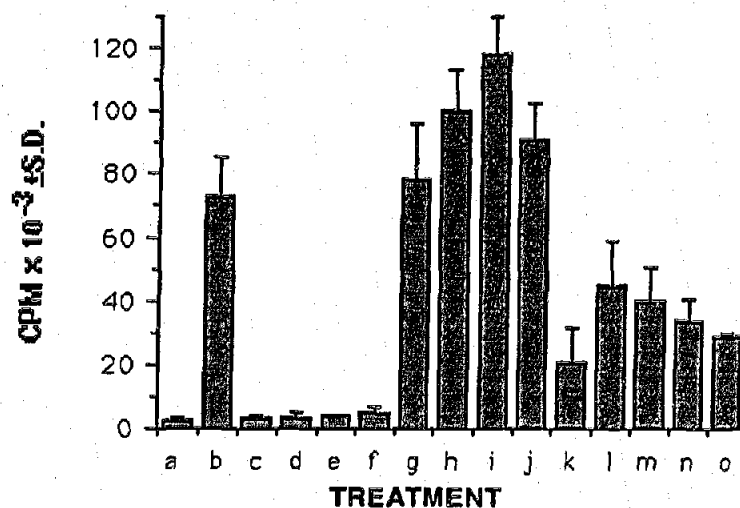


Fig. 2

|                           |  |  |
|---------------------------|--|--|
| a=Nil                     | f=hrIL-1 $\beta$ 50 $\mu$ g/ml                 | k=hrIL-1ra 250ng/ml + Con A 10 $\mu$ g/ml          |
| b=Con A 10 $\mu$ g/ml     | g=hrIL-1 $\beta$ 400ng/ml+ Con A 10 $\mu$ g/ml | l=hrIL-1ra 250ng/ml hrIL-1 $\beta$ 400ng/ml+Con A  |
| c=hrIL-1 $\beta$ 400ng/ml | h=hrIL-1 $\beta$ 200ng/ml+ Con A 10 $\mu$ g/ml | m=hrIL-1ra 250ng/ml +hrIL-1 $\beta$ 200ng/ml+ConA  |
| d=hrIL-1 $\beta$ 200ng/ml | i=hrIL-1 $\beta$ 100ng/ml+ Con A 10 $\mu$ g/ml | n=hrIL-1ra 250ng/ml+hrIL-1 $\beta$ 100ng/ml+ ConA  |
| e=hrIL-1 $\beta$ 100ng/ml | j=hrIL-1 $\beta$ 50ng/ml + Con A 10 $\mu$ g/ml | o=hrIL-1ra 250ng/ml +hrIL-1 $\beta$ 50ng/ml + ConA |

Fig. 2. This graph represents the c.p.m. of <sup>3</sup>H-TdR incorporation by lymphocytes stimulated (bars: b, g, h, i, j, k, l, m, n, o) and non-stimulated (bars: a, c, d, e, f) with Con A for 72 h. Bars c, d, e, f (no Con A) and bars l, m, n, o (with Con A) were pretreated for 2 h with IL-1ra. Bars g, h, i, j and l, m, n, o were treated simultaneously with hrIL-1 $\beta$  (different conc.) + Con A. Bar 'a' is untreated cells. Bar 'b' is treated only with Con A and bar 'k' is pretreated for 2 h with IL-1ra and then added Con A. The data are representative of 4 experiments and the values represent the mean  $\pm$  SD of triplicate determinations.

for 2 h with hrIL-1ra and then treated with the mitogen Con A (10  $\mu$ g/ml). This effect is dose-dependent with a maximum inhibition at hrIL-1ra (250 ng/ml) and minimum at hrIL-1ra (2.5 pg/ml). Repeated trials have demonstrated that hrIL-1ra inhibits lymphocyte activation and proliferation. The time-course action of hrIL-1ra is shown in Table I. The first part of this table shows the optimal mitogenic effect of Con A by using 3 different concentrations (2, 10 and 50  $\mu$ g/ml). In the second part of Table I we show that when the PBMC are preincubated for 2 h, or overnight with hrIL-1ra (250 ng/ml) there is a strong and significant inhibitory effect of DNA synthesis. A little less inhibition was obtained with 30 min pretreatment. While, when cells are treated 2 h after Con A, or 6 h before the end of culture, together with <sup>3</sup>H-TdR, to exclude the possible interference of hrIL-1ra on thymidine incorporation, there was no inhibition of mitogenesis.

In another set of experiments, the PBMC cultures were exposed to Con A (10  $\mu$ g/ml), hrIL-1 $\beta$  at different concentrations (400, 200, 100 and 50 ng/ml); hrIL-1 $\beta$  + Con A; hrIL-1ra + Con A and hrIL-1ra + hrIL-1 $\beta$  at different concentrations + Con A (Fig. 2). In this figure, we show that Con A stimulates lymphocyte

DNA synthesis, hrIL-1 $\beta$  (at different concentrations) is inactive, while the addition of hrIL-1 $\beta$  plus Con A to the cells increases the lymphocyte blastogenesis, which is maximum at hrIL-1 $\beta$  100 ng/ml, while 400 and 50 ng/ml resulted to be non-significant. The cell pretreatments (2 h) with hrIL-1ra (250 ng/ml), strongly inhibits the enhancing effect of hrIL-1 $\beta$  with Con A.

Fig. 3 shows the lymphocyte population without macrophages (discarded after adherence). In these experiments the lymphocyte (PBMC) response to Con A was very weak owing to the absence of M $\phi$ s, and the addition of hrIL-1ra to Con A did not influence these results; while, when the cells were treated with hrIL-1 and Con A the <sup>3</sup>H-TdR incorporation strongly increased. This last effect was inhibited by the pretreatment of cells for 2 h with hrIL-1ra (250 ng/ml).

In Table II we show the dose-response inhibitory effect of hrIL-1ra on PBMC 2 h preincubations, and then adding Con A (10  $\mu$ g/ml). When the PBMCs were treated with hrIL-2 plus Con A the mitogenesis increased, the effect being strongly inhibited by hrIL-1ra (250 ng/ml). In other experiments we pretreated PBMC ( $2.5 \times 10^6$ ) for 2 h with hrIL-1ra (250 ng/ml) and then added LPS (10 ng/ml) cultured for 24 h (Table III). In

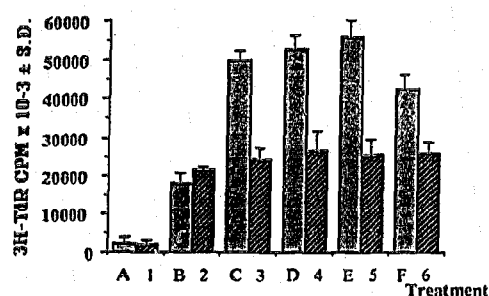


FIG. 3

A=Control  
 B=Con A 10µg/ml  
 C=hrIL-1β 400ng/ml + Con A 10µg/ml  
 D=hrIL-1β 200ng/ml + Con A 10µg/ml  
 E=hrIL-1β 100ng/ml + Con A 10µg/ml  
 F=hrIL-1β 50ng/ml + Con A 10µg/ml  
 1=Control + hrIL-1ra 250ng/ml (no Con A)  
 2=hrIL-1ra 250ng/ml + Con A 10µg/ml  
 3=hrIL-1ra 250ng/ml + hrIL-1β 400ng/ml + Con A 10µg/ml  
 4=hrIL-1ra 250ng/ml + hrIL-1β 200ng/ml + Con A 10µg/ml  
 5=hrIL-1ra 250ng/ml + hrIL-1β 100ng/ml + Con A 10µg/ml  
 6=hrIL-1ra 250ng/ml + hrIL-1β 50ng/ml + Con A 10µg/ml

Fig. 3. The graph represents the c.p.m. of <sup>3</sup>H-TdR incorporation by a lymphocyte population without macrophages, discarded by adherence, stimulated with Con A for 72 h. The cells are pretreated with IL-1ra (columns: 3–6) and columns C–F are not pretreated with hrIL-1ra. To both groups were added hrIL-1β plus Con A. Column A = untreated cells; column 1 = pretreated with only hrIL-1ra; column B = treated only with Con A; and column 2 = pretreated with hrIL-1ra then with Con A (after 2 h). The data are representative of 4 experiments and the values represent the mean ± SD of triplicate determinations.

these experiments hrIL-1ra completely abolished LPS-induced IL-1α generation.

Each figure and table presented in this paper are representative of 4 experiments using 4 different healthy donors.

#### 4. DISCUSSION

Recently it has been reported that the role of antigen-activated macrophages in monokine production and T-cell activation is critical to the host's orchestration of the immune response [8]. Moreover, it is well known that antigens stimulate macrophages to generate IL-1, which is an important early signal for the activation of resting T-cells to become antigen-specific T-cells, capable of expressing the IL-2 receptor, producing soluble IL-2 and secreting other lymphokines such as IL-4, IL-6, interferon-γ (IFN-γ), etc. [20]. The exact mechanism of IL-1 production and regulation by macrophages is still somewhat of an enigma. However, the latest results reported in the immunological literature make it clear that hrIL-1ra, a cytokine also released by Mφs, down-regulate IL-1 production by human monocytes.

In our study, we show that lymphocyte blastogenesis, induced by the mitogen Con A is inhibited by hrIL-1ra in a dose-dependent manner. This effect is most probably due to the inhibitory effect of hrIL-1ra on macrophage IL-1 production, necessary for T-cell activation. Our results are in accordance with Arend et

Table II

| Treatment  | <sup>3</sup> H-TdR incorporation (c.p.m.) ± SD | P <   |
|--|--|-------|
| Nil  | 1150 ± 380                                     | —     |
| hrIL-1ra (no Con A)  | 1063 ± 401                                     | —     |
| hrIL-2 (no Con A)  | 9560 ± 2019                                    | —     |
| Con A (10 µg/ml)   | 54280 ± 1560                                   | (*)   |
| hrIL-1ra (250 ng/ml) + Con A (10 µg/ml)                      | 26150 ± 1320                                   | 0.001 |
| hrIL-1ra (25 ng/ml) + Con A (10 µg/ml)                       | 29820 ± 1112                                   | 0.001 |
| hrIL-1ra (2.5 ng/ml) + Con A (10 µg/ml)                      | 32988 ± 1091                                   | 0.001 |
| hrIL-1ra (0.25 ng/ml) + Con A (10 µg/ml)                     | 38147 ± 1667                                   | 0.01  |
| hrIL-1ra (0.025 ng/ml) + Con A (10 µg/ml)                    | 46321 ± 2153                                   | 0.05  |
| hrIL-1ra (0.0025 ng/ml) + Con A (10 µg/ml)                   | 52686 ± 2667                                   | N.S.  |
| hrIL-2 (1000 U/ml) + Con A (10 µg/ml)                        | 90750 ± 3410                                   | 0.001 |
| hrIL-1ra (250 ng/ml) + hrIL-2 (1000 U/ml) + Con A (10 µg/ml) | 50825 ± 5991                                   | N.S.  |

[<sup>3</sup>H]Thymidine incorporation and mean ± SD in human PBMC after exposure to Con A (10 µg/ml), hrIL-1ra at different concentrations (2 h before Con A or hrIL-2), alone or in combination, and hrIL-2 alone and in combination with Con A and with hrIL-1ra + Con A, during 72 h incubation time. P values (Student's *t*-test) are calculated by comparing hrIL-1ra + Con A, hrIL-2 + Con A or hrIL-1ra + hrIL-2 + Con A-treated cells with Con A (\*) alone

al., who found that hrIL-1ra exhibits a dose-responsive inhibition of IL-1α and IL-1β augmentation of PHA-induced murine lymphocyte proliferation [12]. In addition, these authors found that hrIL-1ra is a strong inhibitor of PGE2. Since there is evidence that prostaglandins, in physiological concentrations, are implicated in many cellular control processes and may play an important role in lymphocyte stimulation in vitro (being that PGs stimulate adenylcyclase activity and markedly increase the cAMP levels) [21,22], it is also possible that the down-regulation of PGE2 by hrIL-1ra may contribute to the inhibition of lymphocyte blastogenesis.

In this study, the suppression by hrIL-1ra pretreated peripheral blood mononuclear cells on mitogen-stimulated proliferation was maximum at 250 ng/ml, the highest concentration tested. However, we did not test higher concentrations of IL-1ra on the basis of

Table III

| Treatment                             | IL-1α (pg/ml) ± SD |
|---------------------------------------|--------------------|
| Control                               | 109 ± 96           |
| LPS (10 ng/ml)                        | 4546 ± 801         |
| hrIL-1ra (250 ng/ml)                  | 115 ± 86           |
| hrIL-1ra (250 ng/ml) + LPS (10 ng/ml) | 175 ± 98           |

Human PBMC (2.5 × 10<sup>6</sup> cells/ml) in 3 healthy donors (mean of 3 experiments) pretreated or not with hrIL-1ra (2 h) and then activated with LPS (10 ng/ml) for 24 h at 37°C, 5% CO<sub>2</sub> (ELISA).

physiological reasoning. Moreover, we show that the inhibitory effect of hrIL-1ra on mitogenesis is maximum when the PBMC were preincubated with this monocyte product for 2 h. When monocytes were discarded from the entire population of PBMC and only the lymphocytes were cultured, the treatment with Con A was effective only when exogenous hrIL-1 was added at different concentrations. This effect was partially inhibited by 2 h pretreatment of hrIL-1ra, suggesting that hrIL-1ra down-regulated not only endogenous IL-1 released by macrophages but also antagonizes the exogenous hrIL-1.

The restorative effects exerted by hrIL-2 after the inhibition of hrIL-1ra demonstrate that the block of IL-1 by hrIL-1ra consequently leads to the lack of production of IL-2, an important growth factor for T-cells. Moreover, the reversibility of hrIL-1ra's action on the cells indicate a non-toxic effect, probably involving the down-regulation of the gene expression through the inhibition of mRNA of IL-1. Since the activation of T cells involves IL-1 as the first signal in the production of other cytokines, such as the growth factor IL-2 the inhibition of mitogen stimulated lymphocyte DNA synthesis by hrIL-1ra is most probably due to the inhibition of interleukin-1 as is shown in Table III. However the activation of T cells has not been fully elucidated. There are different points of view regarding IL-1's ability to stimulate T cells. The prominent belief is that IL-1 is a factor that promotes mitogen-driven T cell blastogenesis [16]. Pereira, Miller and Shevach reported two alternative pathways for T cell activation: one pathway is cyclosporin-resistant and IL-2 independent; the second is cyclosporin-sensitive, cytokine dependent (including IL-2), postulating the existence of a new T cell growth factor released by T lymphocytes [26]. Recently, Mizel reported that CD4 T cells may be divided into two subpopulations, TH1 and TH2, which are both activated by mitogen presenting cells and produce different interleukins: TH1 release IL-2, IL-3 and IFN $\gamma$  which activates macrophages, while TH2 respond to the presented antigen and IL-1 to release IL-3, IL-4 and IL-6 [27].

The existence of IL-1 inhibitor (IL-1i) was previously described by Dinarello et al. in 1982 which demonstrated the existence of a circulating thymocyte suppressor factor during endotoxic fever in humans. IL-1i blocks the binding of IL-1 to its receptors on T-cells but does not block the binding of TNF $\alpha$  or IL-2 [23,24]. It is possible that IL-1ra and IL-1i are the same factor. In fact, these two compounds both had a potent effect on T cell activation and proliferation as confirmed by our studies.

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